Efficient Recovery of 3D Forces exerted by Cells on Thin Substrates

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The quantification of cellular forces provides insight into the way cells sense and react to the extracellular matrix (ECM) in a variety of physiological and pathological processes. Recent so-called 2.5D Traction Force Microscopy (TFM) studies have revealed the importance of normal forces exerted by cells lying on 2D gels, emphasizing the 3D nature of cell forces.

Boussinesq Green analytical function has been widely used to recover cell forces when the gel is approximated by an elastic semi-infinite medium. However, its applicability is limited to a minimum gel thickness for which the half-space approximation still holds. Alternatively, the Finite Element Method (FEM) can be used to recover the 3D traction forces of adherent cells on thin gels. An invertible stiffness matrix can be built from a number of Green functions obtained by applying unit tractions in each Cartesian direction to each facet in the surface of the meshed substrate and solving the forward problem for the stress equilibrium [1]. However, the large volumetric grids (~10,000 elements in the surface) required to get acceptable values of sensitivity and resolution affect the computational efficiency, which, ultimately, can limit the applicability of FEM-based solutions to large experimental data sets.

Here, we present an alternative FEM-based methodology for 2.5D TFM experiments on thin gels that provides an efficient and flexible way to recover 3D cell forces.

Assuming the cell is located far enough from the lateral boundaries of the substrate, the Green function only depends on relative displacements of the spatial points and not on the absolute location of the applied unit tractions, i.e. it can be considered shift-invariant. Therefore, we can build the FEM-based stiffness matrix from a single Green function (per Cartesian direction) and a number of shifted copies of it, instead of calculating the Green function for every node on the substrate surface. Furthermore, the number of required shifts and, thus, the size of the stiffness matrix that has to be inverted, directly depends on the number of spatial locations where cell forces would be expected, which typically is limited to the gel surface covered by the cell body. However, an unsuitable imaging of the cell could lead to a biased force recovery. Instead, we can constrain the calculation of the cell forces to the surroundings of the local maxima of the displacement field, avoiding the need of labeling the cell body or other structures such as focal adhesions.

Abaqus CAE 6.12 has been used to mesh the substrate and apply a unit traction at the center of its top surface. The resulting Green function was adaptively sampled (finer mesh closer to the force application point) to allow a subsequent interpolation step in Matlab, which allows reusing the same Green function for different data sets as long as the mechanical properties of the material remain unaltered. The areas where forces are calculated have been obtained by thresholding the magnitude of the displacements at the gel surface.

Our results show similar resolutions and sensitivities than previous methodologies [1] with a dramatic improvement in the computational time, which allows a fast recalculation of the stiffness matrix for TFM studies under different mechanical conditions.

[1] Legant WR, Choi CK, Miller JS, Shao L, Gao L, Betzig E, Chen CS. 2013. Multidimensional traction force microscopy reveals out-of-plane rotational moments about focal adhesions. Proc Natl Acad Sci U S A. 110:8816

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